**CDI+/CDI- competition with different ratios and different cell densities**

Day 1 (7/29/2014)

-Start o/n cultures of inhibitor and target strains carrying fluorescent markers

-Inoculate one colony of inhibitor and target, separately, into tubes containing 2ml of LB respectively

-Grow overnight at 37°C at 200 rpm shaking

Day 2 (7/30/2014)

*Morning*

Mix inhibitor and target at different ratios (1:1, 1:100, 1:10000, inhibitor to target ratio)) and check ratios by viable count

|  |  |  |  |
| --- | --- | --- | --- |
| 1ul of each gives: | o/n culture inhibitor | Plate dilutions from | |
|  |  | Kan | Amp |
| o/n culture of inhibitor | 1:1 ratio | -4, -3 | -4, -3 |
| 100xdilution of inhibitor | 1:100 ratio | -4, -3 | -2, -1 |
| 10000xdilution of inhibitor | 1:10000 ratio | -4, -3 | -1, 0 |

**Viable counts (when needed or wanted):** Prepare 4 eppendorf tubes with 450ul of PBS for each of the mixes that you just prepared. Make serial dilutions of each of the mixes in PBS, use 50ul for each transfer to get a 10-fold dilution in each step. Plate 100ul from the marked dilutions on LB-plates containing respective antibiotic.

Inoculate the different ratios at different density to start a 10 generations growth (9 conditions repeated 2 times):

- Make a dilution series of the o/n cultures of inhibitor and target respectively as follows (preparation of 1 mL of 100x dilutions with 10uL inside 990uL)

- Solution 1:

Preparation of 10mL diluted 1000x solutions of each ratios (5uL of CDI+ non diluted/diluted 100x/diluted 10000x and 5uL of CDI- non diluted inside 10mL LB)

-Take 2mL of solution 1 in a glass tube to start the growth from density 10^6 bacteria per mL to 10^9 (density 1)

- Solution 2:

Dilute 25uL of solution 1 in 2475uL of LB (100x dilution of solution 1)

-Take 2mL of solution 2 in a glass tube to start the growth from density 10^4 bacteria per mL to 10^7 (density 2)

- Solution 3:

Dilute 20uL of solution 2 in 1980uL of LB (100x dilution of solution 2) in a glass tube to start the growth from density 10^2 bacteria per mL to 10^5 (density 3)

*5 hours later*

-Prepare the cultures to monitor ratios of the cultures by running them on the flow cytometer (see protocol for Flow cytometry) and by plating for viable counts (if no signal on flow cytometry)

* Density 10^6 to 10^9: 10uL into 190uL of filtered PBS
* Density 10^4 to 10^7: spin 1mL of undiluted culture and resuspend into 200uL of filtered PBS, then dilute it 10x (50uL into 450uL of filtered PBS) to get less than a 2000 events per seconds in the flow cytometer
* Density 10^2 to 10^5: spin 1mL of undiluted culture and resuspend into 200uL of filtered PBS

*Rk: for next time,*

*-look at each undiluted solution to estimate size of clumps*

*-do vortex each solution before dilution in PBS to dissolve clumps in order to run the flow cytometer count*

**Flow cytometry:** Take 1ml of the mixes of inhibitor and target that you just prepared. Spin down cells for 3min at 6000rpm. Remove supernatant and resuspend pellet in 200ul of sterile filtered PBS. Run samples on the flow cytometer.

- Freeze at -80°C the 500uL remaining in glycerol 25% (mix with 500uL of glycerol 50%).

Day 3 / 4 / 5

*Morning*

-Passage 2ul of each o/n culture into 2ml of fresh LB

*5 hours later*

-Flow cytometer count

-Freeze at -80°C